PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

DUBUC, Jean, H.
Goudreau Gage Dubuc
Stock Exchange Tower
Suite 3400, 800 Place Victoria
P.O. Box 242
Montreal, Quebec H4Z 1E9
CANADA

Date of mailing (day/month/year)
04 October 2000 (04.10.00)

Applicant's or agent's file reference
CG/10875.93

IMPORTANT NOTIFICATION

International application No.
PCT/CA00/00047

International filing date (day/month/year)
19 January 2000 (19.01.00)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
DUBUC, Jean, H.
Goudreau Gage Dubuc & Martineau Walker
The Stock Exchange Tower
Suite 3400, 800 Place Victoria
P.O. Box 242
Montreal, Quebec H4Z 1E9
Canada

State of Nationality:
State of Residence:
Telephone No.: 514 397 7604
Facsimile No.: 514 397 4382
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
DUBUC, Jean, H.
Goudreau Gage Dubuc
Stock Exchange Tower
Suite 3400, 800 Place Victoria
P.O. Box 242
Montreal, Quebec H4Z 1E9
Canada

State of Nationality:
State of Residence:
Telephone No.: 514 397 7604
Facsimile No.: 514 397 4382
Teleprinter No.:

3. Further observations, if necessary:
The indication of a new company's name of the agent on the Demand (Form PCT/IPEA/401) has been considered a request for recording a change under Rule 92bis. In case of disagreement, the International Bureau should be notified immediately.

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer
F. Baechler

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 04 October 2000 (04.10.00)	
International application No. PCT/CA00/00047	**Applicant's or agent's file reference** CG/10875.93
International filing date (day/month/year) 19 January 2000 (19.01.00)	**Priority date** (day/month/year) 19 January 1999 (19.01.99)
Applicant PAQUIN, Bruno et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

18 August 2000 (18.08.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer F. Baechler Telephone No.: (41-22) 338.83.38

CA0000047

Form PCT/IB/331 (July 1992)

WHAT IS CLAIMED IS:

1. A process for generating an oligonucleotide library which originates from a chosen biological material, comprising:

a) generating random oligonucleotides, wherein said oligonucleotides are of a uniform length comprising a central segment of randomly varied bases and segments of a defined sequence flanking the central segment on each side;

b) hybridizing said random oligonucleotide mixture of a) with a nucleic acid template of biological origin under hybridization conditions which enable the formation of duplexes, while minimizing or abrogating mismatches;

c) separating said duplexes from non-duplexed material; and

d) amplifying said hybridized oligonucleotides.

2. The process of claim 1, wherein said template is genomic DNA or cDNA.

3. The process of claim 1 or 2, wherein said biological material is selected from genomic DNA and cDNA.

4. A method of subtracting between different oligonucleotide libraries which contain common sequence motifs comprising:

a) partitioning double stranded OL under denaturing conditions to separate: (i) antisense strands hybridized with template and (ii) sense strands;

b) annealing said antisense strand of OL1 with an excess of OL2 sense strand, under hybridization conditions;

c) partitioning double stranded hybrids (OL1 antisense/ OL2 sense) and single stranded OL2 sense molecules from single stranded antisense OL1 molecules;

d) amplifying OL1 antisense molecules; and

e) repeating steps a) to d) until OL1 antisense does not have complementary sequences to OL2 sense.

5. The method of claim 4, wherein said partitioning is carried out using streptavidin/biotin.

6. A process for generating an oligonucleotide library which originates from a chosen biological material, comprising:

a) generating a random oligonucleotide mixture, wherein said oligonucleotides are of a uniform length;

b) hybridizing said random oligonucleotide mixture of a) with a nucleic acid template of biological origin under hybridization conditions which enable the formation of duplexes, while minimizing or abrogating mismatches;

c) separating said duplexes from non-duplexed material;

d)amplifying said hybridized oligonucleotides; and

e) subtracting between different oligonucleotide libraries which contain common sequence motifs.

7. The process of claim 6, wherein said subtracting between different oligonucleotide libraries which contain common sequence motifs comprises:

a) partitioning double stranded OL under denaturing conditions to separate: (i) antisense strands hybridized with template and (ii) sense strands;

b) annealing said antisense strand of OL1 with an excess of OL2 sense strand, under hybridization conditions;

c) partitioning double stranded hybrids (OL1 antisense/ OL2 sense) and single stranded OL2 sense molecules from single stranded antisense OL1 molecules;

d) amplifying OL1 antisense molecules; and

e) repeating steps a) to d) until OL1 antisense does not have complementary sequences to OL2 sense.

8. The process of claim 7, wherein said partitioning is carried out using streptavidin/biotin.

9. The process of any one of claims 6 to 8, wherein said template is genomic DNA or cDNA.

10. The process of any one of claims 6 to 9, wherein said biological material is selected from genomic DNA and cDNA.

11. An oligonucleotide library produced by the process of any one of claims 1, 2, 3, 6, 7, 8, 9 or 10.

12. Use of the oligonucleotide library of claim 11 in a diagnostic kit.

13. Method of diagnosis comprising use of the oligonucleotide library of claim 11.

14. Use of the oligonucleotide library of claim 11 in reversed dot blots wherein said oligonucleotide library is covalently bound to membranes.

15. Use of the oligonucleotide library in claim 11, wherein said oligonucleotide library is hybridized against oligonucleotide chip arrays.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
CG/10875.93		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00047	19/01/2000	19/01/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant UNIVERSITE DE MONTREAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/08/2000	29.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Favre, N Telephone No. +49 89 2399 7363

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00047

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-21 as originally filed

Claims, No.:

1-14 with telefax of 10/01/2001

Drawings, sheets:

1/7-7/7 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 8-14.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 8-14 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☒ the claims, or said claims Nos. 8-14 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 1-7

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-7
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations
see separate sheet

R Item I

Basis of the report

Some amendments filed with the letter dated 10.01.2001 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following:

1. The deletion of the expression "from a chosen **biological** material" and the addition of the term "synthetic" in claim 1 and in claim 4 (former claim 6) extends the scope of the claims to biological **and synthetic** material. Although the application as filed referred to nucleic acids **representative** of genomes (title and page 1, lines 9-11), the use of synthetic DNA or RNA for the claimed methods was not **specifically** disclosed in the application as originally filed, which only further referred to material from biological origin. It is well accepted that the disclosure of specific examples does not enable the use of a general term, as said general term encompasses more than the specific examples disclosed. Thus, the addition of the term "synthetic" extends the scope of the claims beyond the content of the application as filed.

1.1 The same objection applies for the amendments of dependent claim 6, which refers to **synthetic** DNA, cDNA or RNA.

1.2 Moreover, claim 6 refers to RNA in general, whereas the original disclosure only refers to "nucleic acids", DNA, cDNA and expressed mRNA (page 1, line 11 of the description) but does however not **specifically** refer to RNA. The addition of "RNA" in the wording of claim 6 thus extends the scope of said claims beyond the content of the application as filed.

2. Novel claim 4 refers to ranges of **10-40** bases for the central segments and flanking segments. Said ranges were not **specifically** disclosed in the application as originally filed and thus extend beyond the content of the application as filed.

3. According to the argumentation of point 1. above, the fact that the application as filed refers to membranes (former claim 14) and magnetic beads (page 13, lines 9-25), does not enable the introduction of the general expression "solid support" in independent claim 13. Thus the use of the expression "solid support" in independent claim 13 is considered to be a generalisation which extends beyond the content of the application as originally filed

3.1 Moreover, the addition in dependent claim 14 of **specific** solid supports, i.e. glass slide, coated glass slide, printed arrays, microspheres and chromatographic media, which were not originally **specifically** disclosed is considered to extend beyond the content of the application as filed.

Therefore, the above-mentioned amendments have not been taken in consideration for the purposes of this written opinion.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 8 defines an oligonucleotide library **obtainable** using the methods of claims 1-7. Claims 9-14 define particular uses of said library of double-stranded oligonucleotides **obtainable** using the methods of claims 1-7.
Claims for products defined in terms of a process of manufacture are admissible only if the products as such fulfil the requirements for patentability, i.e. *inter alia* that they are new and inventive. A product is not rendered novel merely by the fact that it is produced by means of a new process.

Characteristic for a library obtained using the methods of claims 1-7 is that the oligonucleotides are double-stranded, of **any** (predetermined) uniform length, and that said oligonucleotides consist of a random (unknown) central part flanked on each sides by a region with a known sequence, e.g. primer sequences or adapter sequences.

A population of PCR products obtained using an error-prone polymerase, or one "band" obtained in a RDA system, are encompassed within the broad wording claims 8-14 under given circumstances. Moreover, under given circumstances, a population of unknown DNA generated with restriction enzymes and having a uniform length also falls within the scope of claims 8-14, when said population is ligated into a known vector, i.e. known flanking sequences. Hence, said DNA populations have the same technical characteristics than a library of oligonucleotides obtained using the method of claims 1-7

It is therefore not possible to distinguish a library of oligonucleotides directly obtained using the method of claims 1-7 from any other library having the same technical characteristics.

Therefore, the scope of generic claims 8-14 is considered to be too broad and thus not supported by the description in the sense of Article 6 PCT.

Moreover, claims 8-14 are also not considered to be sufficiently disclosed in the application as filed for the skilled person to carry them out and thus do not meet the requirements of Article 5 PCT.

Pursuant to Article 34(4)(a)(ii) PCT and in the light of the above objections, it is not possible to assess claims 8-14 for novelty and inventive step in the sense of Articles 33(2), (3) and 33(4) PCT.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The document "Nucleic Acids Research, 2000, **28**(2):605-609" indicated in the search report as a P-document is not to be regarded as state of the art according to Article 33(2) PCT, as the date of priority claimed can be allowed for the relevant

parts of the present application (Article 8 PCT).

2. Document D1 (WO-A-96 34875), which is considered to represent the most relevant state of the art, discloses (cf. page 15, lines 4-31) a method for generating a library of oligonucleotides which are specific for a target. Said target can be a nucleic acid molecule (page 10, lines 20-28).

Document D2 (BioTechniques, 1998, **25**:434-438) refers to representational difference analysis (RDA). For the generation of DP2, DP1 (a population of random nucleotides having adapters on both ends and having an uniform length within each band) is hybridised with a target population and only specific duplexes are further amplified (see table 1 and figures 1 and 2).

2.1 However, none of the prior art documents at hand discloses or fairly suggests a method for generating a library of oligonucleotides that are specific for a given set of nucleic acids, wherein said method uses oligonucleotides having a central single-stranded random nucleotide sequence and defined flanking regions blocked by complementary oligonucleotides, as defined in independent claim 1.

2.2 Hence, the subject-matter of independent claim 1 and of dependent claims 2-7, which further define particular embodiments of the method of claim 1, is considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

The demand must be filed directly with the competent International Preliminary Examining Authority or, if two or more Authorities are with the one chosen by the applicant. The full name or two-letter code of that Authority may be indicated by the applicant on the line

IPEA/ _____

PCT

CHAPTER II

DEMAND

under Article 31 of the Patent Cooperation Treaty:
The undersigned requests that the international application specified below be the subject of international preliminary examination according to the Patent Cooperation Treaty and hereby elects all eligible States (except where otherwise indicated).

For International Preliminary Examining Authority use only

Identification of IPEA	Date of receipt of DEMAND

Box No. I IDENTIFICATION OF THE INTERNATIONAL APPLICATION

Applicant's or agent's file reference: KB/10875.93

International application No.	International filing date *(day/month/year)*	(Earliest) Priority date *(day/month/year)*
PCT/CA00/00047	19 January 2000 (19-01-00)	19 January 1999 (19-01-99)

Title of invention
PROCESS FOR THE GENERATION OF OLIGONUCLEOTIDE LIBRARIES (OLS) REPRESENTATIVE OF GENOMES OR EXPRESSED MRNAS (CDNAS) AND USES THEREOF

Box No. II APPLICANT(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
UNIVERSITÉ DE MONTRÉAL
Postal Code 6128,
Station A
Montreal (Quebec)
H3C 3J7
CANADA

Telephone No.: (514) 343-6786
Facsimile No.: (514) 343-2326
Teleprinter No.:

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
CA	CA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
PAQUIN, Bruno
289 Randill
Châteauguay (Quebec)
J6J 2P4
CANADA

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
CA	CA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*
BRUKNER, Ivan
1882 Sherbrooke East #2
Montreal (Quebec)
H2K 1B5
CANADA

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
CA	CA

☒ Further applicants are indicated on a continuation sheet.

International application No.
PCT/CA00/00047

Continuation of Box No. II APPLICANT(S)

If none of the following sub-boxes is used, this sheet is not to be included in the demand.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

TREMBLAY, Guy
3341 Maréchal #4
Montreal (Quebec)
H3T 1M8
CANADA

State *(that is, country)* of nationality:	State *(that is, country)* of residence:
CA	CA

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

State *(that is, country)* of nationality:	State *(that is, country)* of residence:

☐ Further applicants are indicated on another continuation sheet.

International application No.
PCT/CA00/00047

Box No. III AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The following person is ☒ agent ☐ common representative

and ☒ has been appointed earlier and represents the applicant(s) also for international preliminary examination.

☐ is hereby appointed and any earlier appointment of (an) agent(s) /common representative is hereby revoked.

☐ is hereby appointed, specifically for the procedure before the International Preliminary Examining Authority, in addition to the agent(s)/common representative appointed earlier.

Name and address: *(Family name followed by given name; for a legal entity, full official The address must include postal code and name of country.)*

DUBUC, Jean H.; LECLERC, Alain M.; PRINCE, Gaétan; LUPIEN, Marc; BRITT, Katherine
GOUDREAU GAGE DUBUC
Stock Exchange Tower
800 Place Victoria, Suite 3400
P.O. Box 242
Montreal, Quebec H4Z 1E9 CANADA

Telephone No.: (514) 397-7604

Facsimile No.: (514) 397-4382

Teleprinter No.:

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. IV BASIS FOR INTERNATIONAL PRELIMINARY EXAMINATION

Statement concerning amendments:*

1. The applicant wishes the international preliminary examination **to start on the basis of:**

☒ the international application as originally filed.

the description ☒ as originally filed
☐ as amended under Article 34

the claims ☐ as originally filed
☒ as amended under Article 19 (together with any accompanying statement)
☐ as amended under Article 34

the drawings ☒ as originally filed
☐ as amended under Article 34

2. ☐ The applicant wishes any amendment to the claims under Article 19 to be considered as reversed.

3. ☐ The applicant wishes the start of the international preliminary examination **to be postponed** until the expiration of 20 months from the priority date unless the International Preliminary Examing Authority receives a copy of any amendments made under Article 19 or a notice from the applicant that he does not wish to make such amendments (Rule 69.1(d)). *(This check-box may be marked only where the time limit under Article 19 has not yet expired.)*

* Where no check-box is marked, international preliminary examination will start on the basis of the international application as originally filed or, where a copy of amendments to the claims under Article 19 and/or amendments of the international application under Article 34 are received by the International Preliminary Examining Authority before it has begun to draw up a written opinion or the international preliminary examination report, as so amended.

Language for the purposes of international preliminary examination: ENGLISH

☒ which is the language in which the international application was filed.
☐ which is the language of a translation furnished for the purposes of international search.
☐ which is the language of publication of the international application.
☐ which is the language of the translation (to be) furnished for the purposes of international preliminary examination.

Box No. V ELECTION OF STATES

The applicant hereby **elects all eligible States** *(that is, all States which have been designated and which are bound by Chapter II of the PCT)*

excluding the following States which the applicant wishes **not to elect:**

International application No.
PCT/CA00/00047

Box No. VI CHECK LIST

The demand is accompanied by the following elements, in the language referred to in Box No. IV, for the purposes of international preliminary examination:

			For International Preliminary Examining Authority use only: received	not received
1. translation of international application	:	0 sheets	☐	☐
2. amendments under Article 34	:	0 sheets	☐	☐
3. copy (or where required, translation) of amendments under Article 19	:	4 sheets	☐	☐
4. copy (or, where required, translation) of statement under Article 19	:	2 sheets	☐	☐
5. letter	:	0 sheets	☐	☐
6. other *(specify)*	:	0 sheets	☐	☐

The demand is also accompanied by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ nucleotide and or amino acid sequence listing in computer readable form
6. ☐ other *(specify)*:

Box No. VII SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the demand).

GOUDREAU GAGE DUBUC

By: Katherine Britt

KATHERINE BRITT

For International Preliminary Examining Authority use only

1. Date of actual receipt of DEMAND:
2. Adjusted date of receipt of demand due to CORRECTIONS under Rule 60.1(b):
3. ☐ The date of receipt of the demand is AFTER the expiration of 19 months from the priority date and item 4 or 5, below, does not apply. ☐ The applicant has been informed accordingly.
4. ☐ The date of receipt of the demand is WITHIN the period of 19 months from the priority date as extended by virtue of Rule 80.5.
5. ☐ Although the date of receipt of the demand is after the expiration of 19 months from the priority date, the delay in arrival is EXCUSED pursuant to Rule 82.

For International Bureau use only

Demand received from IPEA on:

CHAPTER II

PCT

FEE CALCULATION SHEET

Annex to the Demand for international preliminary examination

International application No.	PCT/CA00/00047
Applicant's or agent's file reference	KB/10875.93

For International Preliminary Examining Authority use only

Date stamp of the IPEA

Applicant
UNIVERSITÉ DE MONTRÉAL et al.

Calculation of prescribed fees

1. Preliminary examination fee	2,998.29	P
2. Handling fee *(Applicants from certain States are entitled to a reduction of 75% of the handling fee. Where the applicant is (or all applicants are) so entitled, the amount to be entered at H is 25% of the handling fee.)*	287.51	H
3. Total of prescribed fees Add the amounts entered at P and H and enter total in the TOTAL box	3,285.80	TOTAL

Mode of Payment

☐	authorization to charge deposit account with the IPEA (see below)	☐	cash
☐	cheque	☐	revenue stamps
☐	postal money order	☐	coupons
☒	bank draft	☐	other *(specify)*:

Deposit Account Authorization *(this mode of payment may not be available at all IPEAs)*

The IPEA/ _____ ☐ is hereby authorized to charge the total fees indicated above to my deposit account.

☐ *(this check-box may be marked only if the conditions for deposit accounts of the IPEA so permit)* is hereby authorized to charge any deficiency or credit any overpayment in the total fees indicated above to my deposit account.

Deposit Account Number _____ Date *(day/month/year)* _____ Signature _____

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* CG/10875.93

Box No. I TITLE OF INVENTION

Process for the generation of oligonucleotide libraries (OLs) representative of genomes or expressed mRNAs (cDNAs) and uses thereof

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

UNIVERSITÉ DE MONTRÉAL
Postal Code 6128,
Station A
Montreal, Quebec
H3C 3J7
CANADA

☐ This person is also inventor.

Telephone No.
(514) 343-6786

Facsimile No.
(514) 343-2326

Teleprinter No.

State *(that is, country)* of nationality: CA

State *(that is, country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

PAQUIN, Bruno
289 Randill
Châteauguay, Quebec
J6J 2P4
CANADA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: CA

State *(that is, country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DUBUC, Jean H.; LECLERC, Alain M.; PRINCE, Gaétan; LUPIEN, Marc
GOUDREAU GAGE DUBUC & MARTINEAU WALKER
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Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTOR(S)

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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Montreal, Quebec
H2K 1B5
CANADA

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: CA

State *(that is, country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

TREMBLAY, Guy
3341 Maréchal #4
Montreal, Quebec
H3T 1M8
CANADA

This person is:
- ☐ applicant only
- ☒ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: CA

State *(that is, country)* of residence: CA

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
- ☐ applicant only
- ☐ applicant and inventor
- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☒ **AP** ARIPO Patent: GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA** Eurasian Patent: AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP** European Patent: AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA** OAPI Patent: BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☒ AE United Arab Emirates
- ☒ AL Albania .....
- ☒ AM Armenia .....
- ☒ AT Austria .....
- ☒ AU Australia .....
- ☒ AZ Azerbaijan
- ☒ BA Bosnia and Herzegovina .....
- ☒ BB Barbados
- ☒ BG Bulgaria .....
- ☒ BR Brazil .....
- ☒ BY Belarus .....
- ☒ CA Canada
- ☒ CH and LI Switzerland and Liechtenstein
- ☒ CN China .....
- ☒ CU Cuba .....
- ☒ CZ Czech Republic .....
- ☒ DE Germany .....
- ☒ DK Denmark .....
- ☒ EE Estonia .....
- ☒ ES Spain .....
- ☒ FI Finland .....
- ☒ GB United Kingdom
- ☒ GD Grenada
- ☒ GE Georgia .....
- ☒ GH Ghana .....
- ☒ GM Gambia
- ☒ HR Croatia .....
- ☒ HU Hungary .....
- ☒ ID Indonesia
- ☒ IL Israel .....
- ☒ IN India .....
- ☒ IS Iceland
- ☒ JP Japan .....
- ☒ KE Kenya .....
- ☒ KG Kyrgyzstan .....
- ☒ KP Democratic People's Republic of Korea .....
- ☒ KR Republic of Korea .....
- ☒ KZ Kazakhstan .....
- ☒ LC Saint Lucia
- ☒ LK Sri Lanka
- ☒ LR Liberia
- ☒ LS Lesotho .....
- ☒ LT Lithuania
- ☒ LU Luxembourg
- ☒ LV Latvia
- ☒ MD Republic of Moldova .....
- ☒ MG Madagascar .....
- ☒ MK The former Yugoslav Republic of Macedonia .....
- ☒ MN Mongolia
- ☒ MW Malawi .....
- ☒ MX Mexico .....
- ☒ NO Norway
- ☒ NZ New Zealand .....
- ☒ PL Poland .....
- ☒ PT Portugal .....
- ☒ RO Romania
- ☒ RU Russian Federation .....
- ☒ SD Sudan
- ☒ SE Sweden
- ☒ SG Singapore
- ☒ SI Slovenia .....
- ☒ SK Slovakia .....
- ☒ SL Sierra Leone .....
- ☒ TJ Tajikistan .....
- ☒ TM Turkmenistan .....
- ☒ TR Turkey .....
- ☒ TT Trinidad and Tobago .....
- ☒ UA Ukraine .....
- ☒ UG Uganda .....
- ☒ US United States of America .....
- ☒ UZ Uzbekistan .....
- ☒ VN Viet Nam .....
- ☒ YU Yugoslavia .....
- ☒ ZA South Africa .....
- ☒ ZW Zimbabwe .....

☒ TZ TANZANIA
☒ MA MOROCCO

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☒ COSTA RICA CR .....
☒ DOMINICA DM .....

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 19 January 1999	2,259,745	CA		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): (1)

** Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA/

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country (or regional Office)

Box No. VIII CHECK LIST: LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	:	4
description (excluding) sequence listing part)	:	21
claims	:	4
abstract	:	1
drawings	:	6
sequence listing part of description	:	
Total number of sheets	:	36

This international application is **accompanied** by the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as items(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☐ other *(specify):*

Figure of the drawings which should accompany the abstract: 1

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

GOUDREAU GAGE DUBUC & MARTINEAU WALKER

By: _____

Jean H. DUBUC

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA/
6. ☐ Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

DUBUC, Jean H.
GOUDREAU GAGE DUBUC
Stock Exchange Tower
800 Place Victoria, Suite 3400
P.O. Box 242
Montreal, Quebec H4Z 1E9
CANADA

PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL PRELIMINARY
EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	29.03.2001
Applicant's or agent's file reference CG/10875.93	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00047	19/01/2000	19/01/1999

Applicant
UNIVERSITE DE MONTREAL et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Papiol Rovira, M Tel.+49 89 2399-7199

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
CG/10875.93		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/CA00/00047	19/01/2000	19/01/1999
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant UNIVERSITE DE MONTREAL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
18/08/2000	29.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Favre, N Telephone No. +49 89 2399 7363

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/CA00/00047

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-21	as originally filed	

Claims, No.:

1-14	with telefax of	10/01/2001

Drawings, sheets:

1/7-7/7	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application.

   ☒ claims Nos. 8-14.

because:

   ☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):

   ☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 8-14 are so unclear that no meaningful opinion could be formed (*specify*):
   see separate sheet

   ☒ the claims, or said claims Nos. 8-14 are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the standard.

   ☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

   Novelty (N) Yes: Claims 1-7

	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-7
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-7
	No:	Claims	

2. Citations and explanations
see separate sheet

R Item I

Basis of the report

Some amendments filed with the letter dated 10.01.2001 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following:

1. The deletion of the expression "from a chosen **biological** material" and the addition of the term "synthetic" in claim 1 and in claim 4 (former claim 6) extends the scope of the claims to biological **and synthetic** material. Although the application as filed referred to nucleic acids **representative** of genomes (title and page 1, lines 9-11), the use of synthetic DNA or RNA for the claimed methods was not **specifically** disclosed in the application as originally filed, which only further referred to material from biological origin. It is well accepted that the disclosure of specific examples does not enable the use of a general term, as said general term encompasses more than the specific examples disclosed. Thus, the addition of the term "synthetic" extends the scope of the claims beyond the content of the application as filed.

1.1 The same objection applies for the amendments of dependent claim 6, which refers to **synthetic** DNA, cDNA or RNA.

1.2 Moreover, claim 6 refers to RNA in general, whereas the original disclosure only refers to "nucleic acids", DNA, cDNA and expressed mRNA (page 1, line 11 of the description) but does however not **specifically** refer to RNA. The addition of "RNA" in the wording of claim 6 thus extends the scope of said claims beyond the content of the application as filed.

2. Novel claim 4 refers to ranges of **10-40** bases for the central segments and flanking segments. Said ranges were not **specifically** disclosed in the application as originally filed and thus extend beyond the content of the application as filed.

3. According to the argumentation of point 1. above, the fact that the application as filed refers to membranes (former claim 14) and magnetic beads (page 13, lines 9-25), does not enable the introduction of the general expression "solid support" in independent claim 13. Thus the use of the expression "solid support" in independent claim 13 is considered to be a generalisation which extends beyond the content of the application as originally filed

3.1 Moreover, the addition in dependent claim 14 of **specific** solid supports, i.e. glass slide, coated glass slide, printed arrays, microspheres and chromatographic media, which were not originally **specifically** disclosed is considered to extend beyond the content of the application as filed.

Therefore, the above-mentioned amendments have not been taken in consideration for the purposes of this written opinion.

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claim 8 defines an oligonucleotide library **obtainable** using the methods of claims 1-7. Claims 9-14 define particular uses of said library of double-stranded oligonucleotides **obtainable** using the methods of claims 1-7.
Claims for products defined in terms of a process of manufacture are admissible only if the products as such fulfil the requirements for patentability, i.e. *inter alia* that they are new and inventive. A product is not rendered novel merely by the fact that it is produced by means of a new process.

Characteristic for a library obtained using the methods of claims 1-7 is that the oligonucleotides are double-stranded, of **any** (predetermined) uniform length, and that said oligonucleotides consist of a random (unknown) central part flanked on each sides by a region with a known sequence, e.g. primer sequences or adapter sequences.

A population of PCR products obtained using an error-prone polymerase, or one "band" obtained in a RDA system, are encompassed within the broad wording claims 8-14 under given circumstances. Moreover, under given circumstances, a population of unknown DNA generated with restriction enzymes and having a uniform length also falls within the scope of claims 8-14, when said population is ligated into a known vector, i.e. known flanking sequences. Hence, said DNA populations have the same technical characteristics than a library of oligonucleotides obtained using the method of claims 1-7

It is therefore not possible to distinguish a library of oligonucleotides directly obtained using the method of claims 1-7 from any other library having the same technical characteristics.

Therefore, the scope of generic claims 8-14 is considered to be too broad and thus not supported by the description in the sense of Article 6 PCT.

Moreover, claims 8-14 are also not considered to be sufficiently disclosed in the application as filed for the skilled person to carry them out and thus do not meet the requirements of Article 5 PCT.

Pursuant to Article 34(4)(a)(ii) PCT and in the light of the above objections, it is not possible to assess claims 8-14 for novelty and inventive step in the sense of Articles 33(2), (3) and 33(4) PCT.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The document "Nucleic Acids Research, 2000, **28**(2):605-609" indicated in the search report as a P-document is not to be regarded as state of the art according to Article 33(2) PCT, as the date of priority claimed can be allowed for the relevant

parts of the present application (Article 8 PCT).

2. Document D1 (WO-A-96 34875), which is considered to represent the most relevant state of the art, discloses (cf. page 15, lines 4-31) a method for generating a library of oligonucleotides which are specific for a target. Said target can be a nucleic acid molecule (page 10, lines 20-28).

   Document D2 (BioTechniques, 1998, **25**:434-438) refers to representational difference analysis (RDA). For the generation of DP2, DP1 (a population of random nucleotides having adapters on both ends and having an uniform length within each band) is hybridised with a target population and only specific duplexes are further amplified (see table 1 and figures 1 and 2).

2.1 However, none of the prior art documents at hand discloses or fairly suggests a method for generating a library of oligonucleotides that are specific for a given set of nucleic acids, wherein said method uses oligonucleotides having a central single-stranded random nucleotide sequence and defined flanking regions blocked by complementary oligonucleotides, as defined in independent claim 1.

2.2 Hence, the subject-matter of independent claim 1 and of dependent claims 2-7, which further define particular embodiments of the method of claim 1, is considered to be novel and inventive in the sense of Articles 33(2) and 33(3) PCT.

WHAT IS CLAIMED IS:

1. A process for generating a library of oligonucleotides that are specific for a given set of nucleic acids, comprising:

a) generating random oligonucleotides, wherein said oligonucleotides are of a uniform length comprising a single-stranded, central segment of randomly varied bases and flanking segments of defined sequences on each side of said central segment;

b) hybridizing the random oligonucleotides of step a) with a nucleic acid-containing template of biological or synthetic origin under hybridization conditions that enable the formation of duplexes and using blockers to avoid hybridization of said flanking segments;

c) eliminating non-specific duplexes formed in step b) using conditions that minimize or abrogate mismatches;

d) separating the hybridized oligonucleotides from the duplexes obtained in step c); and

e) amplifying the oligonucleotides obtained in step d).

2. A process as defined in claim 1, further comprising the step of

f) subtracting between two different oligonucleotide libraries (OL1 and OL2) which contain similar sequence motifs.

3. A process as defined in claim 2, wherein said subtracting in step f) consists in:

a) Generating single stranded versions of OL1 and OL2;

b) annealing the OL1 strands with an excess of OL2 strands, under hybridization conditions;

c) partitioning double stranded hybrids (OL1:OL2) and single stranded OL2 from single stranded OL1;

d) amplifying the single stranded OL1 obtained from step c); and

e) repeating steps a) to d) to obtain OL1 oligonucleotides with reduced affinity for OL2.

4. A process as defined in any one of claims 1 to 3, wherein said central segment comprises 10-40 bases and each one of said flanking segments comprises 10-40 bases.

5. A process as defined in claim 4, wherein said central segment comprises 20 bases and each one of said flanking segments comprises 20 bases.

6. A process as defined in any one of claims 1 to 3, wherein the template of step b) contains at least one of genomic or synthetic DNA or RNA, or cDNA.

7. A process as defined in claim 3, wherein said partitioning is carried out using streptavidin and biotin.

8. A library of oligonucleotides produced by the process of any one of claims 1 to 7.

9. Use of a library of oligonucleotides produced by the process of any one of claims 1 to 7 in a diagnostic kit.

10. Use of a library of oligonucleotides produced by the process of any one of claims 1 to 7 to inhibit gene function.

11. A method of diagnosis comprising use of a library of oligonucleotides produced by the process of any one of claims 1 to 7.

12. Use of a library of oligonucleotides produced by the process of any one of claims 1 to 7 wherein said oligonucleotides are bound to a solid support.

13. A use as defined in claim 12, wherein said solid support is at least one of a membrane, glass slide, coated glass slide, printed arrays, microspheres or chromatographic media.

14. Use of a library of oligonucleotides produced by the process of any one of claims 1 to 7, wherein said oligonucleotides are hybridized to nucleic acid arrays.

LN

PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
CG/10875.93		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/CA 00/ 00047	19/01/2000	19/01/1999
Applicant		
UNIVERSIT DE MONTR AL et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

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[ ] furnished subsequently to this Authority in written form.

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[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

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2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[ ] None of the figures.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 00/00047

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 15651 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUNTER (GB)) 16 April 1998 (1998-04-16) the whole document	1-15
Y	WO 98 15649 A (CREMER THOMAS ;CRAIG JEFF (DE); UNIV HEIDELBERG (DE)) 16 April 1998 (1998-04-16) the whole document	1-15
Y	SEDLACEK ET AL.: "Direct selection of DNA sequences conserved between species" NUCLEIC ACIDS RESEARCH, vol. 21, no. 15, 1993, pages 3419-3425, XP002138409 the whole document	1-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2000	09/06/2000

Name and mailing address of the ISA	Authorized officer
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Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED T BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	ARMOUR ET AL.: "MEASUREMENT OF LOCUS COPY NUMBER BY HYBRIDISATION WITH AMPLIFIABLE PROBES" NUCLEIC ACIDS RESEARCH, vol. 28, no. 2, 2000, pages 605-609, XP002138423 cited in the application the whole document	1-15

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

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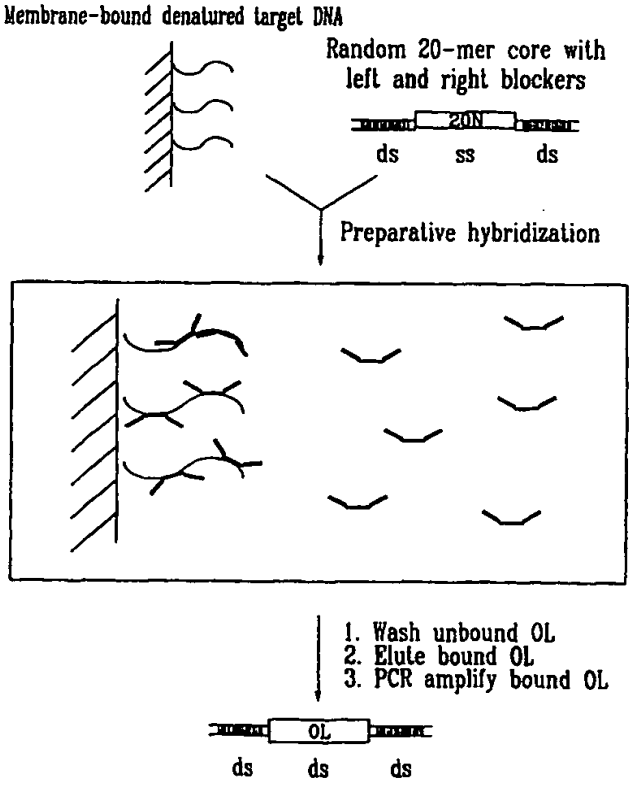
(54) Title: PROCESS FOR THE GENERATION OF OLIGONUCLEOTIDE LIBRARIES (OLs) REPRESENTATIVE OF GENOMES OR EXPRESSED mRNAs (cDNAs) AND USES THEREOF

(57) Abstract

A process for the generation of oligonucleotide libraries representative of a given template is described. Starting from a random pool of oligonucleotides, the process selects only those which hybridize to the template nucleic acid. This selection yields a highly specific library that represents an oligo–image of the chosen template. The novel quality of this approach is the generation of amplifiable oligonucleotide probes that are of uniform length, free of repetitive sequence motifs and easily subjected to differential selection. This technique is used to produce different oligonucleotide libraries (OLs) and shows that these OLs do not cross–hybridize. Differential selection of these OLs produces oligonucleotides that can be used in the identification, characterization and isolation of nucleic acids.



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TITLE OF THE INVENTION

Process for the Generation of Oligonucleotide Libraries (OLs) Representative of Genomes or Expressed mRNAs (cDNAs) and Uses Thereof

FIELD OF THE INVENTION

The present invention relates to a process for the generation oligonucleotide librairies (OLs) representative of genomes or expressed mRNAs (cDNAs) and to the uses thereof. In particular, the present invention relates to a process for the generation of oligonucleotide librairies comprising oligonucleotides of uniform length. The present invention further relates to the uses of these OLs in numerous biotechnological applications, including the identification and/or characterization of biological materials, clinical diagnosis (DNA/RNA level), preparative extraction of specific mRNA (and genes) and genomic research/mapping.

BACKGROUND OF THE INVENTION

The generation of genomic DNA libraries, or cDNA libraries and the maintenance, and handling of these libraries are critical procedures in the field of genomics and/or biotechnology. In classical libraries the relevant segments of DNA are cloned into vectors, which are maintained and propagated in particular biological systems (*in vivo*). Alternatively, libraries (*in vitro*) can be directly constructed from genomic DNA or cDNA. They contain linkers at the 5' and 3' ends of the DNA which allow PCR amplification of the library. The information stored in these libraries

contains repetitive sequence elements that originated from repetitive DNA, or high copy mRNAs. This results in a significant redundancy, which can complicate the use and the outcome of using classical libraries. Another important feature which reduces the utility of classical libraries is the heterogeneity in size of the members comprising the library. This limits the usefulness of classical libraries in subtractive hybridization procedures (1-2) which are dependent upon the length, complexity and the redundancy of the libraries, and which therefore are particularly sensitive to the choice of method and the number of cycles performed. In fact, one must tailor the hybridization conditions to accommodate the heterogeneous length and redundancy of stored information in order to perform subtraction. Thus, the results are more "laboratory-specific" than library-specific.

A number of diagnostic methods that involve nucleic acid hybridization have arisen in recent years. Most of them are designed to provide qualitative information about the presence of a specific sequence motif in a complex analytical mixture of nucleic acids and use a detection system based on PCR and/or DNA chip hybridization technologies (3-7). For both of these technologies, diagnostic oligonucleotides constitute an essential part of the detection system. These oligonucleotides are primarily chosen based on the sequence data of the nucleic acids to be detected. In spite of the power of hybridization to correctly identify a complementary strand, it does face limitations. In fact, the difference in stability between a perfectly matched complement and a complement mismatched at only one base can be as little as 0.5° C (8). This is the fundamental limitation to the power of DNA hybridization for specific identification of a cognate strand. Therefore, the diagnostic power of any chosen oligonucleotide must be validated using an analytical mixture whose sequence context is

not totally known. The problem of adequate probe selection is time and labour-consuming. On the other hand, the growing complexity of detection systems based on oligonucleotide technologies requires a fast selection of a large number of short oligonucleotides.

Akopyants *et al* (7) performed subtractive hybridization using bacterial DNAs digested by high-frequency restriction enzymes. The use of such restriction enzymes tends to generate DNA fragments having a broadly similar size, about 500 base pairs. However, the uniformity is not rigorous. Moreover, the library created by these restriction fragments still contains a significant number of redundant sequences; consequently, patches of short polymorphism embedded in homologous sequences are going to be missed when such a library is used.

U.S. Patent No. 5,270,163 (8) teaches a method for the isolation of nucleic acids using high-affinity nucleic acid ligands. This method has been termed the SELEX method (Systematic Evolution of Ligands by Experimental Enrichment) and is based on the use of proteins or small molecules, but not nucleic acids, as targets. The selection of oligonucleotides in the SELEX method relies on the three-dimensional (3D) shape of the oligonucleotides and their fit into the structures of the target molecules. In contrast to this, the selection of oligonucleotides in the present invention is based on hybridization with target nucleic acid.

Armour *et al* (11) describes the quantitative recovery of amplifiable probes hybridised to an immobilised target. The amplifiable probes consist of PCR or restriction fragments and their technique is meant to assess the copy number of *loci*.

There thus remains a need for oligonucleotide libraries which allow for the use of uniform hybridization conditions to perform selection and/or subtraction while minimizing or eliminating redundant sequences. Advantageously, these libraries can be used in the selection of highly informative and target-specific probe libraries. The present invention seeks to meet these and other needs.

SUMMARY OF THE INVENTION

The procedure described herein results in the generation and selection of oligonucleotide probes with a high specificity for a given system. These oligonucleotides cover the entire length of the target DNA, thus increasing detectability which might be lost in classical oligo-detection systems due to secondary DNA structure or DNA deletions present in an analyte mixture. At the same time, they present inexpensive variants of a multiplex oligonucleotide-detection approach, since they are not required to be individually synthesized.

More specifically, in accordance with the present invention, there is provided a process for the generation of oligonucleotide libraries, or OLs. The present invention teaches a process for generating OLs from genomic DNAs and cDNAs, and for performing the subtraction of these libraries.

The present invention further teaches OLs which allow the use of hybridization conditions which are controllable and reproducible. In addition, the invention teaches a process for the selection of uniform length OLs which minimizes or eliminates redundant sequences and reduces complexity. The result is the production of highly-informative and target-specific probe libraries.

An object of the present invention is therefore to provide a process for the generation of oligonucleotide libraries comprising OLs of uniform length which are self-amplifiable and easily subjected to subtraction.

Another object of the invention is to provide OLs which are compatible with DNA array technology. Indeed, an array of diverse mixtures of oligonucleotides which show differential hybridization patterns could be the best choice for the next generation of DNA diagnostics.

Other objects, advantages and features of the present invention will become more apparent upon reading of the following non-restrictive description of preferred embodiments thereof, given by way of example only with reference to the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Schematic representation of the experimental procedure for the preparation of OL. Denatured DNA is bound to the membrane and hybridized to the random oligonucleotides library in the presence of blockers. Theses blocking primers disallow the unspecific hybridization of left and right oligonucleotide arms used for PCR amplification of the OL. (ss - single-stranded DNA, ds - double-stranded DNA). Hybridization and PCR amplification of OL are described more particular below, in the Experimental Methods.

Figure 2: Dot blot hybridization of OL targeted against different genomes. The first row represents the dot blot hybridization of random probes with the specified genomic DNA (adenovirus, pBluescript and lambda). The

last row shows dot blot hybridization of mixed adenovirus and lambda-selected OL. The other rows are analytical dot blot hybridizations of selected OLs with each of the genomes indicated. The procedures of preparative and analytical hybridization are described in the Experimental Methods, below.

Figure 3: Specificity and probe distribution of OL generated from adenoviral genome. (**A**) The corresponding genome and adenoviral DNA were run on a 1% agarose gel stained with ethidium bromide. The type of restriction enzyme and DNA are indicated on the top of each gel lane. (**B**) Southern hybridization of the same gel using adenovirus OL as an hybridization probe (see Figure 2, row 2). It should be noted that under the experimental conditions, there was no cross-hybridization with either lambda or human DNA. (**C**) The same membrane was stripped and rehybridized with a OL directed against a 3648 bp-long restriction fragment. This subset of adenovirus OL was prepared by cutting the membrane corresponding to the 3648 bp band from a similar southern blot and reamplified by PCR as described in the Experimental Methods, below. Thus, it is shown that OL specificity may be enhanced by controlling the choice of targeted DNA fragments in the next round of selection.

Figure 4: The distribution of OL along genomic DNA. The densitrometric scan of radioactive signal from OL was integrated over total adenoviral genome (Figure 3, lanes 3 and 5) using Scion Image software (Scion corporation, Frederick, Maryland). The signal intensity of OL probes hybridizing to restriction fragments is linearly proportional to the length of DNA.

Figure 5: Subtractive enrichment of OL. (**A**) The tester OL is presented by the mixture of two genomes (Adenovirus type 2 and Lambda phage). The driver OL was produced from the Lambda genome only. The single stranded (ss) OL from driver DNA was used to pool out the complementary single stranded mixed tester OL. After removing the subtracted fraction, the remainder of the mixed OL was used as a probe in the analytical hybridization step. The mixed OL probes were analyzed by dot blotting as described (**B**) before subtractive enrichment and (**C**) after subtractive enrichment by hybridization to genomic Adenovirus and Lambda DNA.

Figure 6: Relative distribution of 20-mers with the different number of mismatches which do hybridize to targeted DNA. The abscissa shows the number of mismatches present in the 20-mer, while the y-axis illustrates the corresponding relative frequencies. The distribution profile was obtained by calculating the number of combinations for each particular number of mismatches which are thermostable at 52° C. The y-axis was normalized to reflect the relative distribution (%) over the total number of captured oligonucleotides (100%). The majority of n-mers captured after the first round of selection will be 20-mers with less then 6 mismatches. This is described further in the Detailed Description, below.

DETAILED DESCRIPTION

The present invention thus provides a process for the generation of oligonucleotide libraries having the following characteristics:

1) A uniform length of about 60 bases, comprising a central segment of about 20 bases randomly varied to represent all possible combinations,

and segments of about 20 bases of a defined sequence flanking the central segment on each side;

2) A uniform number of copies for each sequence motif (consequently, there are no differential hybridization kinetics which could originate from the presence of repetitive DNA); and

3) A melting profile which is characterized by a sharp transition from double stranded to single stranded (or vice versa) oligonucleotides. This is a critical advantage in subtractive hybridization procedures.

The use of these OLs enhances the specificity of hybridization to nucleic acids isolated from various sources, thereby allowing for the preparation of oligonucleotide mixtures useful in the detection and quantification of specific nucleic acids or nucleic acid mixtures.

In one particular embodiment, the starting pool of oligonucleotides is chemically synthesized and consists of a random region of a fixed length (L), flanked by a constant sequence (primer binding sites, PBS). The random oligonucleotide pool covers n copies (n=1,2,3...) of all sequence combinations of length L, i.e. 4^L, which is a total of 10^{12} different sequence motifs for L=20 nucleotides. The basic length of oligonucleotides is long enough to generate uniform sequence motifs for a particular biological system. The complexity of the library (10^{12}) overcomes the complexity of the template (which is usually between 10^4-10^9). The random pool is then hybridized with a nucleic acid template isolated from any selected source and the unbound oligonucleotides are washed away under stringent conditions. The remaining, template-bound oligonucleotides are then subjected to amplification, using PCR or other methods known to those of skill in the art and using primers complementary to the constant

flanking segments, thus producing a library of oligonucleotides capable of selectively hybridizing to nucleic acid templates.

The choice of 20-mer for the length of the oligonucleotide library is not arbitrary but is based on the rationale that the length of the particular sequence motif should be long enough to be unique for even the most complex genomes. The L-mer of length L will be, on average, repeated every 4 power L base pairs. The longer the L, the greater the average distance between 2 identical sequence motifs of length L will be. However, this particular combinatorial approach is at best approximative, and other lengths may be suitable as well. The choice of length will depend on such factors as the length and/or complexity of the genome to be detected and compatibility with current nucleic acid amplification and DNA array technologies.

In another embodiment, the amplification described above is performed with one of the PCR primers being labelled with biotin, providing means for purification of the labelled products with streptavidin-labelled substrates (12) or other similar methods. The amplified mixture of unlabelled oligonucleotides specific to one template is hybridized with labelled mixtures of oligonucleotides selected for specificity to one or several nucleic acid templates, and the unbound material is collected. In this manner, a mixture of nucleotides which is enriched for nucleic acids present in the unlabelled library only can be generated.

The process is based on stringent hybridization. Furthermore, high fidelity hybridization between pools of oligonucleotides and templates (genomic DNA or cDNA) is the basic mode of transfer of genomic

information into OLs. An efficient subtractive hybridization procedure is used to accommodate the features of the aforementioned OLs.

The present invention is illustrated in further detail by the following non-limiting example.

EXAMPLE 1

Generation of OLs, Use Thereof in Subtractive Hybridization to Generate Subtractive Oligonucleotides Libraries (SOLs), and Use of OLs or SOLs in Hybridization Experiments

EXPERIMENTAL METHODS

DNA / oligonucleotides

The starting random DNA pool was synthesised by GIBCO BRL (Burlington, Canada), (RAN), 5'-GCCTGTTGTGAGCCTCCTGTCGAA-N_{20}-TTGAGCGTTTATTCTTGTCTCCC-3'. The corresponding left and right arms were (LEFT) 5'-GCCTGTTGTGAGCCTCCTGTCGAA-3' and (RIGHT) 5'-BioGGGAGACAAGAATAAACGCTCAA-3'. The 5'-end biotinylated oligonucleotides were used to pool out complement strands, using BioMag magnetic particles (PerSeptive Biosystems, Framingham, MA). During preparative hybridization, the left and right arms were blocked by (LEFT) 5'-TTCGACAGGAGGCTCACAACAGGC-3' and (RIGHT) 5'GGGAGACAAGAATAAACGCTCAA-3'. Theses oligonucleotides are termed 'blockers' in the text.

The following genomic DNA was used to produce OL: Adenovirus DNA Type 2, (GIBCO BRL), Lambda DNA cI857 *ind1 Sam 7* (New England Biolabs), pBluescript II SK(+) (Stratagene, San Diego, CA). The Human HeLa DNA used as one control was from Clontech (Palo Alto, CA).

Blotting genomic DNA

The genomic DNA was denatured 2-3 minutes at 95° C and cooled on ice. The nylon membrane (Hybond-N, Amersham Pharmacia Biotech, Piscataway, NJ) was blotted with 100 ng of denatured genomic DNA, dried for 2 minutes on a hot plate and exposed to UV light for 8 minutes. The prehybridization was done for a minimum of 30 minutes in the hybridization buffer (7% SDS, 0.25M Na2HPO4 pH7.4, 1mM EDTA, pH 8.0 and 10g/L of BSA).

Hybridization and washing of the starting random pool

The preparative hybridization between random core (20N) and targeted DNA was done with 10 pmoles of starting random pool (RAN). The random pool was pre-mixed with 100 pmoles (10 times more than RAN) of LEFT and RIGHT blockers in order to exclude cross-hybridization of left and right arms with genomic DNA. The oligonucleotide mixture was heated up to 95°C, cooled at room temperature and added to the hybridization buffer. The hybridization was done overnight at 50° C. The first washing was done with 6X SSC, followed by subsequent 2X SSC washing at the same temperature as hybridization was done.

Generating OL by PCR

The dot containing the genomic DNA and bound probes was cut out of the nylon membrane (radius of 2-4mm), soaked in 100 µl H_20 and heated to 95°C for 1-2 minutes. The solution containing the denatured probe (originally RAN) was then collected and passed threw a Sephadex G-50 column in order to eliminate salts and SDS. The PCR was prepared under standard conditions, typical for SELEX-like amplification of DNA (10, 13). The RIGHT 5'-end biotinylated primer of the sense strand (the

one which did not hybridise with genomic DNA) and LEFT primer of antisense strand were used in the PCR reaction. The temperature cycles were 53°C, 72°C, 95°C, each 30 seconds, repeated 20 times.

Probe labelling and hybridization

Before labelling, the PCR reaction mixtures were passed threw Sephadex G-50 columns. Around 100-200 ng of PCR product was labelled with 50 pmols of γP^{32} ATP (6000 Ci/mmol, I.C.N. pharmaceuticals, Irvine, CA). The total amount of probe radioactivity was 300 000 c.p.m. The probe was added into 0.5 ml of hybridization buffer. The blotting of genomic DNA was done as described above. Hybridizations were done overnight at 50°C. The nylon membrane was washed as previously described, and exposed to Kodak X-OMAT film.

OL labelling and analytical hybridization

The generated OL was tested, using 1) the original genomic DNA from which they were selected (positive control) and 2) using the unrelated genomic DNA (negative control). The OL labelling, hybridization and probe washing was done as described, except that hybridization time was shorter (60 minutes).

Southern blot hybridization

Electrophoresis was performed in a 1% agarose gel with TBE buffer (80 mM Tris borate, pH 8.0, 2mM Na_2EDTA) and stained with ethidium bromide. One ug of BstEII-digested lambda DNA, 300 ng of adenoviral DNA and 1 µg of AluI-HpaI-digested human HeLa DNA were run on the gel according to specifications (all restriction enzymes used in this work were purchased from New England Biolabs). For Southern hybridization, DNA was transferred to Nylon membranes by capillary blot procedure

following manufacturer's recommendations (Amersham Pharmacia Biotech). Hybridization was performed as described above with adenoviral OL. Autoradiographic exposure (using Kodak X-OMAT film) was done at room temperature, for few hours. Stripping of the membrane was done by boiling a 1% SDS solution and pouring it over the Nylon membrane.

Subtractive enrichment of OL

The tester OL (mixed OL) that reflects the two genomes (Adenovirus type 2 and Lambda) was made by preparing OL from equimolar mixtures of 2 genomes. The driver OL was produced from the lambda genome only. The production of sense strand (the one which did not hybridize with genomic DNA) was done using 5'-end biotinylated primer in PCR reaction. After denaturing PCR product, the biotinylated sense strand was bound to streptavidin magnetic particles (200 µg, binding capacity > 200 pmols of biotinylated oligonucleotides, Biomag Magnetic Particles, PerSeptive Biosystems), and pulled-out using a magnet. The complementary antisense strand was discarded with the liquid phase. The mixed antisense tester OL (Lambda + Adenovirus DNA) was produced in the same way. This time, the supernatant with the antisense, non-biotinylated strand was hybridized overnight at 50°C with 10 times molar excess of driver Lambda sense stand attached to magnetic beads. The hybridization buffer was the same as described above but without SDS. After removing the fraction bound to the magnetic beads, the rest of the mixture was used in the analytical hybridization step.

RESULTS

The starting random pool of oligonucleotides contains 4^{20} (i.e. 10^{12}) different 20-mers. The diversity of the sequence motifs is approximately

10^{11} higher than the diversity of the most complex genomes. A schematic representation of the procedure for generating OL is presented in Figure 1 and is described in detail in the Experimental Methods, above.

Blockers were used in order to avoid hybridization of the flanking arms to the targeted genome, and this step was found to be critical to achieve specificity. The stringency of hybridization conditions eliminates unbound 20-mers, leaving the specific oligonucleotides bound to the membrane via hybridization of the random core to the genome (Fig. 1). This ensemble of selected oligonucleotides constitutes the OL.

It should be noted that the starting random pool of oligonucleotides contained about 8 copies of each sequence motif during the first hybridization step (10-20 pmoles) and that the number of copies of each particular 20-mer present in the random mixture was smaller than the number of genome copies.

Figure 2 shows that OLs are able to discriminate genomes with complexities around 10^3 to 10^4. The starting random pool of probes binds to all three genomes equally (Fig. 2, row 1). After one round of selection, the OL can hybridise specifically towards a single targeted genome (Fig. 2, rows 2, 3 and 4). The OL can be selected against a mixture of two genomes and the specificity is conserved for both genomes (Fig. 2, row 5).

A Southern blot was performed in order to document the distribution of adenovirus OL probes along the genome (Fig. 3). There was no apparent cross-hybridization of adenovirus OL to either HeLa or Lambda DNA (Fig. 3b, lanes 1, 4, 5 and 6). The intensity of radioactive signal over adenoviral genome generated by adenovirus-specific OL was linearly increasing with

the DNA fragments' length (Fig. 4). Therefore, one could deduce a uniform distribution of OL throughout the genomic DNA.

In the next step, only the subset of the adenovirus OL bound to the 3648 bp band in Figure 3b (rows 3 and 6) was reamplified and selected. The membrane was washed from the original probe and hybridised with the 3648 bp subset of the original OL. Figure 3c shows that the specificity of the OL subset is obtained against the 3648 bp band. These data illustrate the successful increase in the specificity and the reduction in the complexity of the original Adeno-specific OL to that of the 3648 bp subset, using just one additional round of selection.

One round of subtractive enrichment between two oligonucleotide libraries was performed as schematized (Fig. 5a). The tester OL reflects the two genomes (adenovirus type 2 and lambda phage). The driver OL was produced from the Lambda genome only. The single stranded (ss) OL from the driver DNA was used to pool out the complementary single stranded, mixed, tester OL. After removing the subtracted fraction, the rest of ssDNA was used as a probe in the analytical hybridization step. The intensity of hybridization signals between Lambda and Adeno genomes, before (Fig. 5b) and after (Figure 5c) one round of subtractive enrichment was shown. It should be noted that further subtraction steps could be performed by changing the sequence design of flanking arms between tester and driver OLs, as suggested by recent developments in subtractive procedures (14).

With reference to Figure 6, the relative distribution of 20-mers with different numbers of mismatches that hybridized to the targeted DNA was predicted. The number of 20-mers (N) with (m) number of mismatches

(m=0, 1, ..., 20) capable of hybridizing to the target sequence were then calculated. First, the number of combinations of 20-mers (C) with the same number of mismatches (m) in the initial random pool of oligonucleotides that are capable of hybridizing to a specific 20-mer motif was calculated. Since each full match could be replaced by 3 different mismatches, the number of combinations must be multiplied by 3^m i.e. $C*3^m$. Finally, these numbers were adjusted to reflect the sequence-dependent termostability of 20-mers with (m =0, 1...20) number of mismatches which hybridised at 52°C using the thermostable fraction of the binomial distribution for each n-mer population. Duplexes of more than 7 mismatches are not observed based on this themostability criterion. Therefore, the majority of 20-mers captured after the first round of selection will harbour less than 6 mismatches.

A process that generates amplifiable DNA oligonucleotide libraries which are specific for a given segment of DNA has been described. This process is akin to random priming, because it is possible to generate probes without *a priori* knowledge of the template sequence. One round of preparative hybridization was enough to produce genome-specific oligonucleotide libraries (Figs. 2 and 3). The OLs were inferred from genomes of complexity of 10^3-10^4.

The process described herein generates probes with high detection power. These probes/selected oligonucleotides can contain mismatches. The notion that introduction of artificial mismatches could increase detection power of oligonucleotides during single nucleotide polymorphism (SNP) detection was well documented by Guo *et al* (6). However, the prediction of positions and types of mismatches, which should be introduced to increase detectability of oligonucleotide, remains

undefined. Consequently, to enhance oligonucleotide detectability by introducing (artificial) mismatches, one must search different positions and types of mismatches along the oligonucletide. Once they are empirically determined, i.e. tested on 2 different sequence motifs, the oligonucleotide containing particular mismatches could be used (15). The present process provides an approach based on differential selection of thermostable oligonucleotides (i.e. their differential stability), which are present in one, but not in the second system. The selection of oligonucleotides with the highest detectability is inherently present in this process, i.e. the method suggests a solution to the problem of where and what type of mismatches should be introduced to increase detection power of oligonucleotide, or to find the particular oligonucleotide which best discriminates between 2 sequence motifs which may differ by a single base.

Without wishing to be bound by any hypothesis, the following provides an explanation of what is believed to be occurring during the process of the present invention. Based on calculations, it is expected that the 20-mers selected in an OL can contain up to 6 mismatches. Nevertheless, specificity toward a given template was achieved, suggesting that the presence of these putative mismatches did not interfere with good discrimination. In other words, mismatch-free hybridization is not critical for differential detection approach; rather, the *relative differences* in the thermodynamical stabilities of the hybridized oligonucleotides appear to be determinative. The present process uses selection of oligonucleotides based on this criterion and therefore provides the possibility of overcoming current technological limitations. In the second and further rounds of selection, the number of 20-mers both in the targeted genome and the probe mixture (OL) could be adjusted. Each new round of

preparative hybridization (Fig.1) and/or subtraction (Fig. 6) could reduce the complexity of OL, by using the excess OL rather than genomic DNA. Therefore, the average number of mismatches for each particular 20-mer will continue to decline until it reaches the sequence-dependent limitation, but not the concentration-dependent limitation.

In summary, OLs are generated from the template DNAs. These OLs are used in subtractive hybridization, for example between genomic or cDNA-based libraries (OL1 and OL2) to make a new Subtractive Oligonucleotide Library (SOL1/2 and/or SOL2/1)), that is/are specific for one system/library but not for the other. Oligonucleotides isolated from such subtractive libraries (SOL) are useful for diagnostic purposes. They can a) directly serve as highly specific hybridization probes or b) they can be tested for PCR-specific differential amplification, specific for one, but not the other biological system.

These libraries (OL or SOL) can be hybridized to oligonucleotide chip arrays in order to obtain a specific hybridization pattern that is useful for diagnostic features: each OL produces an image which is specific for the templated DNA (genome or cDNA). A particular advantage in using OL or SOL instead of genomic/cDNA libraries is that the hybridization signal is not dependent on copy number and distribution of particular sequence motifs. By comparing images of different genomes/cDNA, one can deduce which oligonucleotides are highly specific for a single genome/cDNA, and use this or these oligonucleotide(s) as "genome tags". The oligonucleotides obtained can also be used for specific diagnostic PCR.

OLs or SOLs can be inferred from two biologically relevant systems, like mammalian cells, to detect fine differences in cell cycle, tissue status, viral infection, age/development status etc.

Although the present invention has been described hereinabove by way of a preferred embodiment, it can be modified by one of skill in the art without departing from the spirit and nature of the subject invention, as defined more particularly in the appended claims.

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WHAT IS CLAIMED IS:

1. A process for generating an oligonucleotide library which originates from a chosen biological material, comprising:

a) generating random oligonucleotides, wherein said oligonucleotides are of a uniform length comprising a central segment of randomly varied bases and segments of a defined sequence flanking the central segment on each side;

b) hybridizing said random oligonucleotide mixture of a) with a nucleic acid template of biological origin under hybridization conditions which enable the formation of duplexes, while minimizing or abrogating mismatches;

c) separating said duplexes from non-duplexed material; and

d) amplifying said hybridized oligonucleotides.

2. The process of claim 1, wherein said template is genomic DNA or cDNA.

3. The process of claim 1 or 2, wherein said biological material is selected from genomic DNA and cDNA.

4. A method of subtracting between different oligonucleotide libraries which contain common sequence motifs comprising:

a) partitioning double stranded OL under denaturing conditions to separate: (i) antisense strands hybridized with template and (ii) sense strands;

b) annealing said antisense strand of OL1 with an excess of OL2 sense strand, under hybridization conditions;

c) partitioning double stranded hybrids (OL1 antisense/ OL2 sense) and single stranded OL2 sense molecules from single stranded antisense OL1 molecules;

d) amplifying OL1 antisense molecules; and

e) repeating steps a) to d) until OL1 antisense does not have complementary sequences to OL2 sense.

5. The method of claim 4, wherein said partitioning is carried out using streptavidin/biotin.

6. A process for generating an oligonucleotide library which originates from a chosen biological material, comprising:

a) generating a random oligonucleotide mixture, wherein said oligonucleotides are of a uniform length;

b) hybridizing said random oligonucleotide mixture of a) with a nucleic acid template of biological origin under hybridization conditions which enable the formation of duplexes, while minimizing or abrogating mismatches;

c) separating said duplexes from non-duplexed material;

d)amplifying said hybridized oligonucleotides; and

e) subtracting between different oligonucleotide libraries which contain common sequence motifs.

7. The process of claim 6, wherein said subtracting between different oligonucleotide libraries which contain common sequence motifs comprises:

a) partitioning double stranded OL under denaturing conditions to separate: (i) antisense strands hybridized with template and (ii) sense strands;

b) annealing said antisense strand of OL1 with an excess of OL2 sense strand, under hybridization conditions;

c) partitioning double stranded hybrids (OL1 antisense/ OL2 sense) and single stranded OL2 sense molecules from single stranded antisense OL1 molecules;

d) amplifying OL1 antisense molecules; and

e) repeating steps a) to d) until OL1 antisense does not have complementary sequences to OL2 sense.

8. The process of claim 7, wherein said partitioning is carried out using streptavidin/biotin.

9. The process of any one of claims 6 to 8, wherein said template is genomic DNA or cDNA.

10. The process of any one of claims 6 to 9, wherein said biological material is selected from genomic DNA and cDNA.

11. An oligonucleotide library produced by the process of any one of claims 1, 2, 3, 6, 7, 8, 9 or 10.

12. Use of the oligonucleotide library of claim 11 in a diagnostic kit.

13. Method of diagnosis comprising use of the oligonucleotide library of claim 11.

14. Use of the oligonucleotide library of claim 11 in reversed dot blots wherein said oligonucleotide library is covalently bound to membranes.

15. Use of the oligonucleotide library in claim 11, wherein said oligonucleotide library is hybridized against oligonucleotide chip arrays.

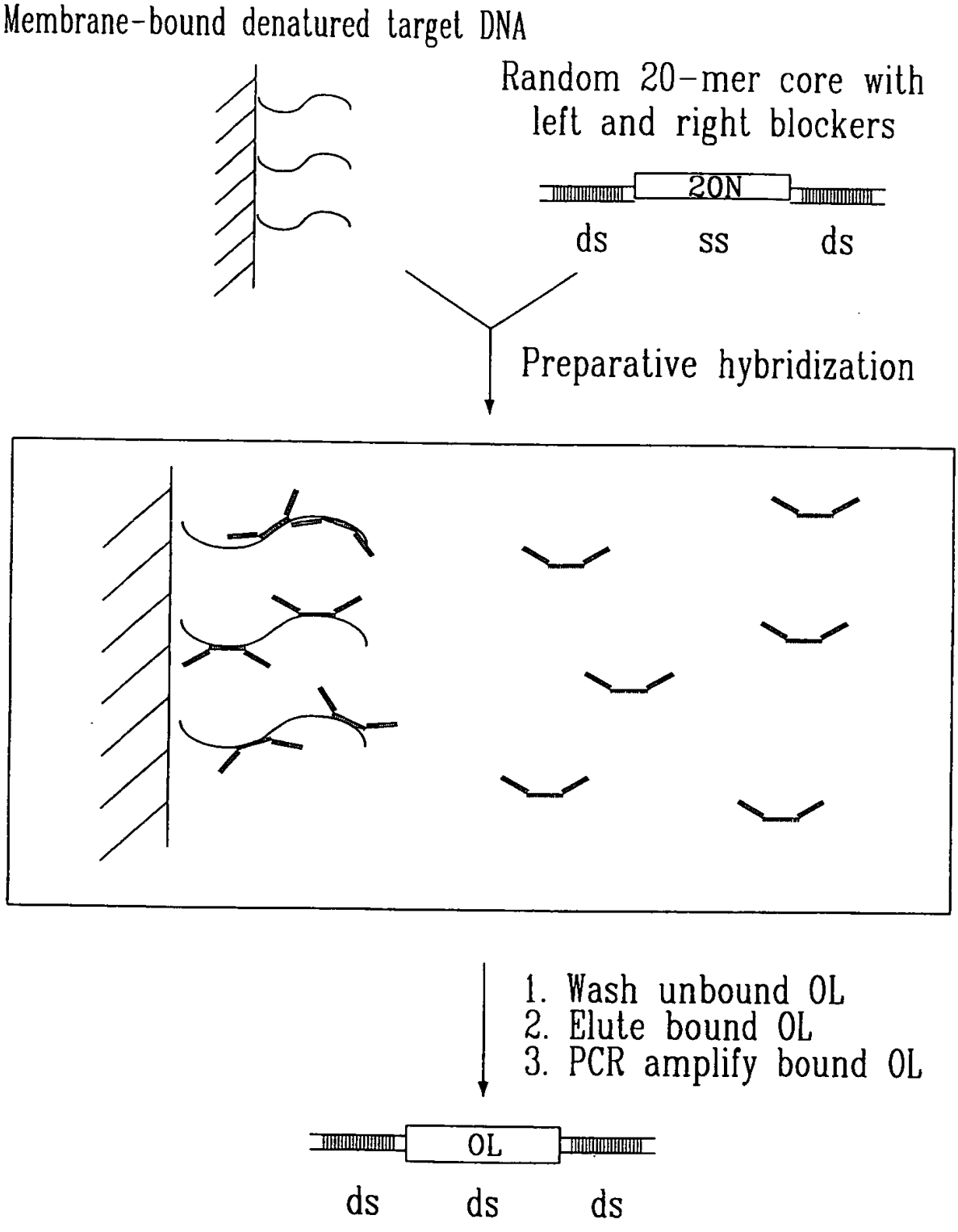
Membrane-bound denatured target DNA
Random 20-mer core with left and right blockers
20N
ds
ss
ds
Preparative hybridization
1. Wash unbound OL
2. Elute bound OL
3. PCR amplify bound OL
OL
ds
ds
ds

Fig. 1

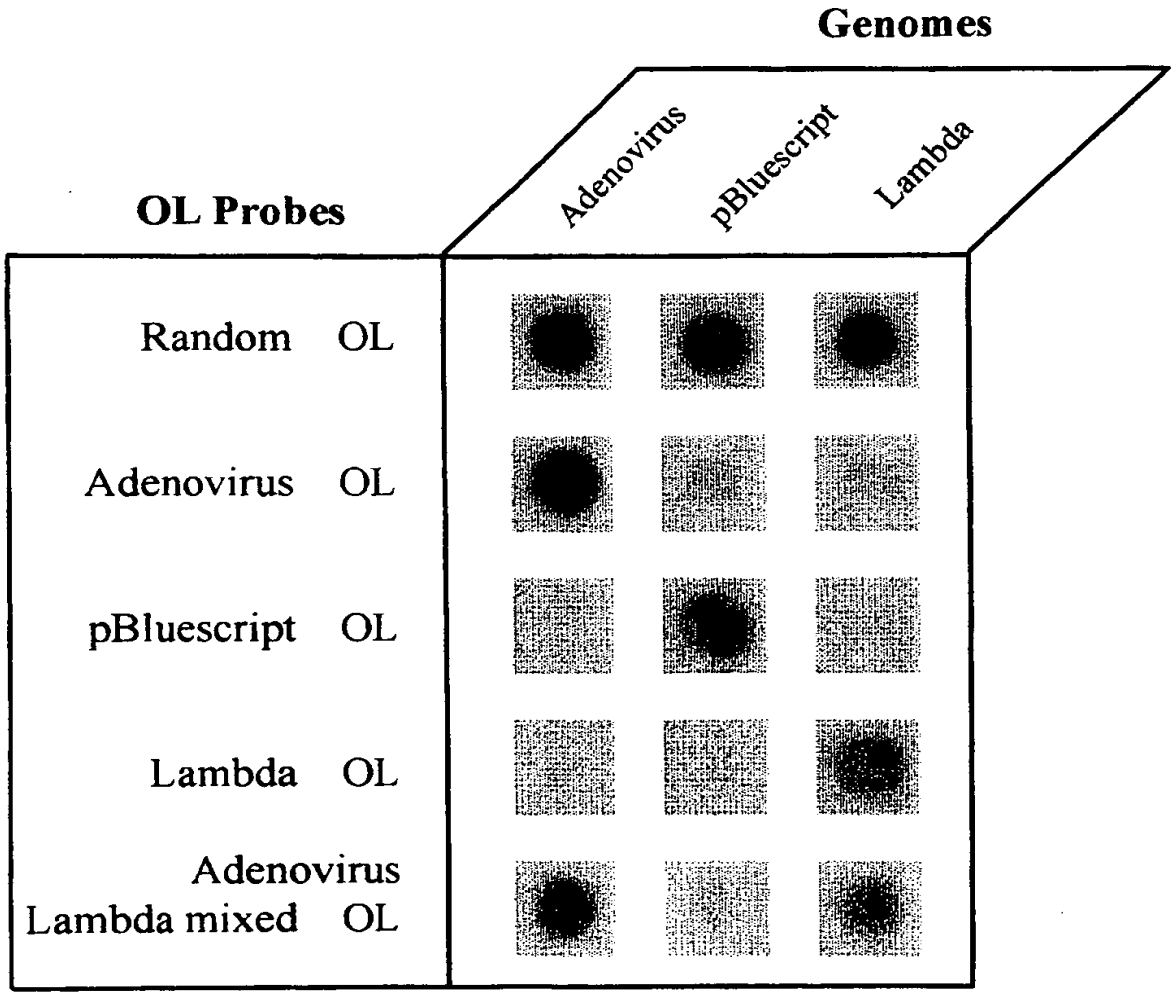
Genomes
Adenovirus
pBluescript
Lambda
OL Probes
Random OL
Adenovirus OL
pBluescript OL
Lambda OL
Adenovirus Lambda mixed OL

FIG 2

FIG - 3A

1 2 3 4 5 6

Lambda BstEII
Adenovirus
Adenovirus KpnI
HeLa DNA AluI + HpaI
Adenovirus & HeLa AluI + HpaI
Adenovirus KpnI & HeLa AluI + HpaI

FIG - 3B

1 2 3 4 5 6

Lambda BstEII
Adenovirus
Adenovirus KpnI
HeLa DNA AluI + HpaI
Adenovirus & HeLa AluI + HpaI
Adenovirus KpnI & HeLa AluI + HpaI

FIG - 3C

1 2 3 4 5 6

Lambda BstEII
Adenovirus
Adenovirus KpnI
HeLa DNA AluI + HpaI
Adenovirus & HeLa AluI + HpaI
Adenovirus KpnI & HeLa AluI + HpaI

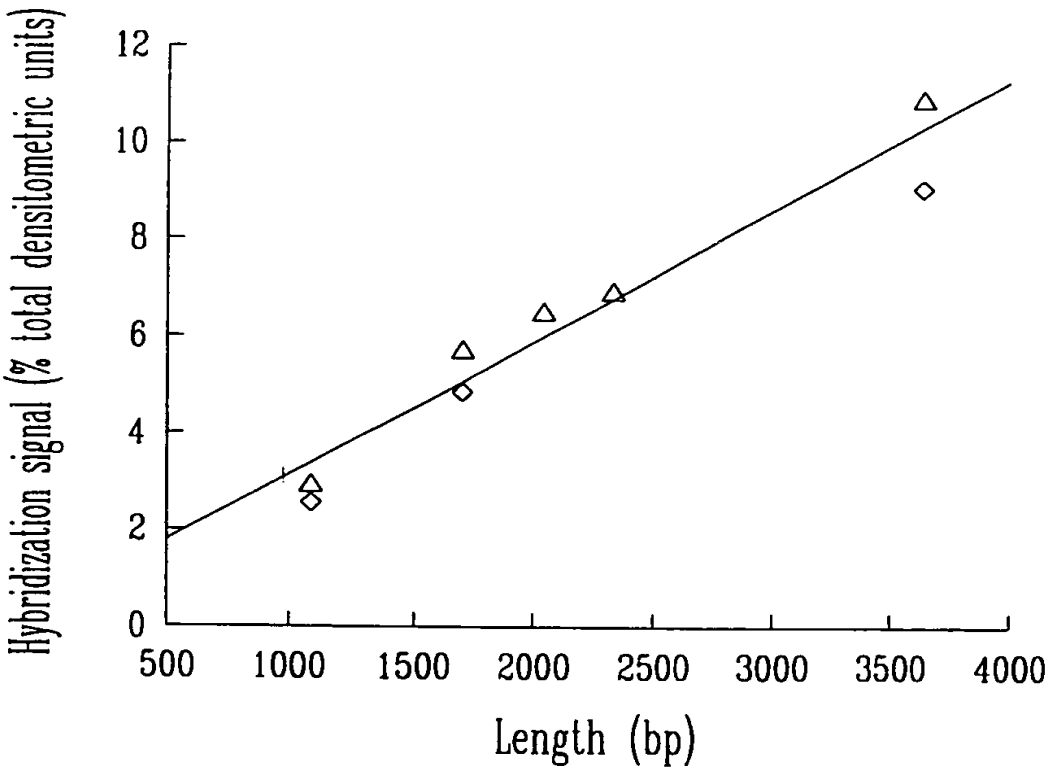
Hybridization signal (% total densitometric units)
12
10
8
6
4
2
0
500
1000
1500
2000
2500
3000
3500
4000
Length (bp)

Fig. 4

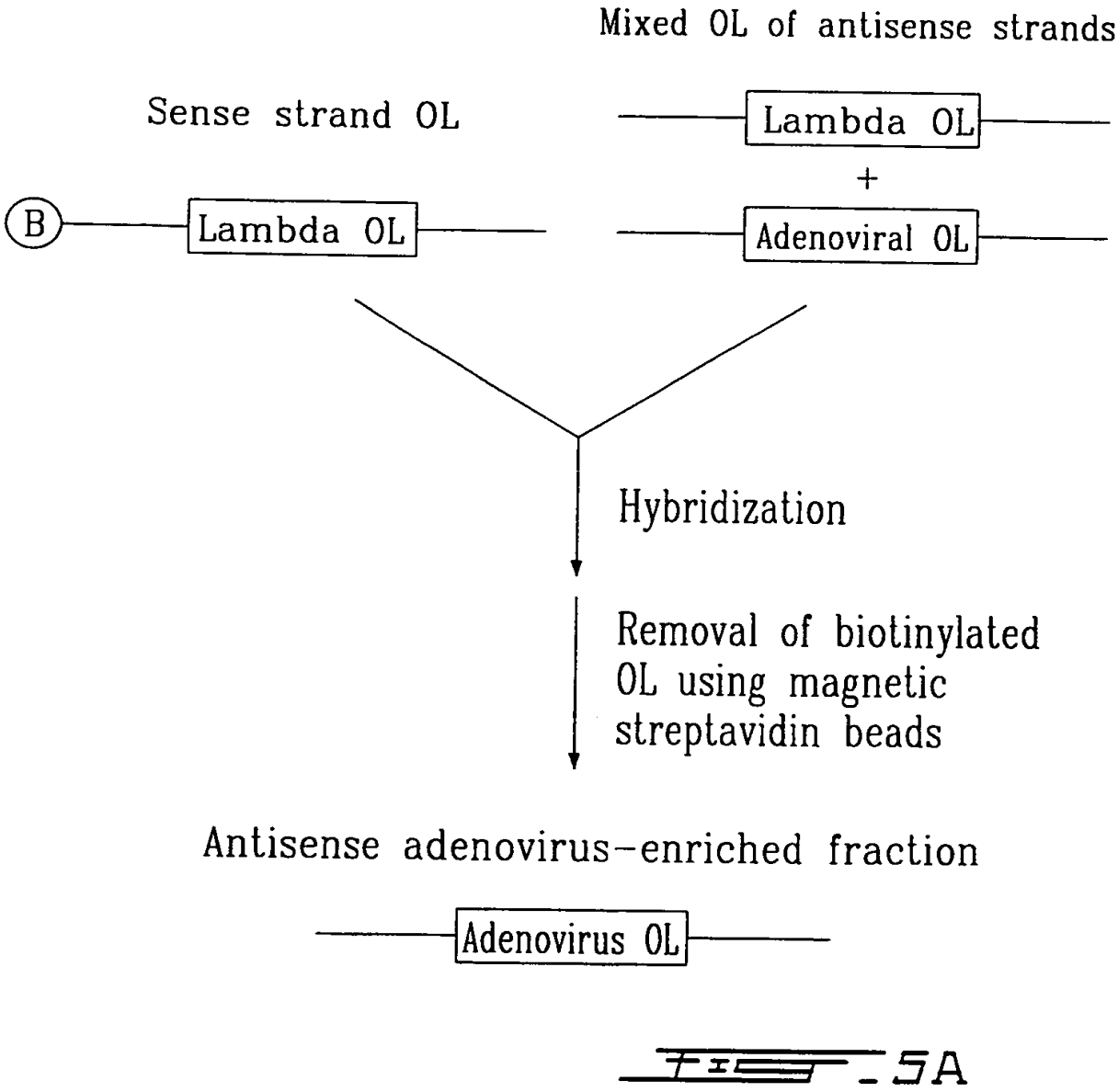
Mixed OL of antisense strands
Sense strand OL
Lambda OL
+
B
Lambda OL
Adenoviral OL
Hybridization
Removal of biotinylated OL using magnetic streptavidin beads
Antisense adenovirus-enriched fraction
Adenovirus OL

FIG 5A

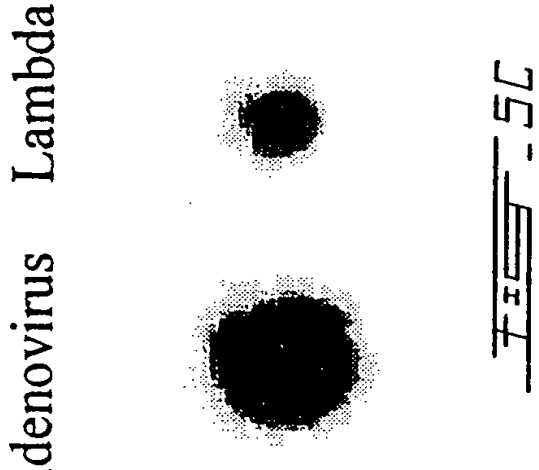

Fig - 5C

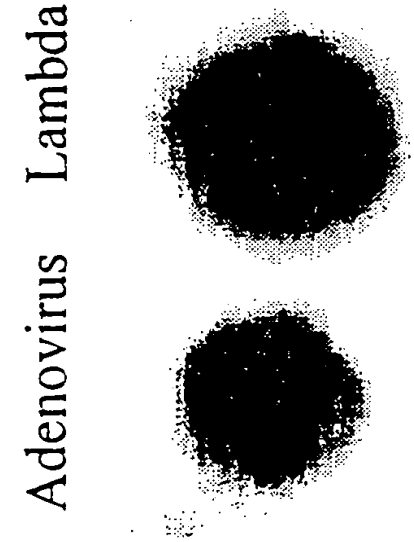

Fig - 5B

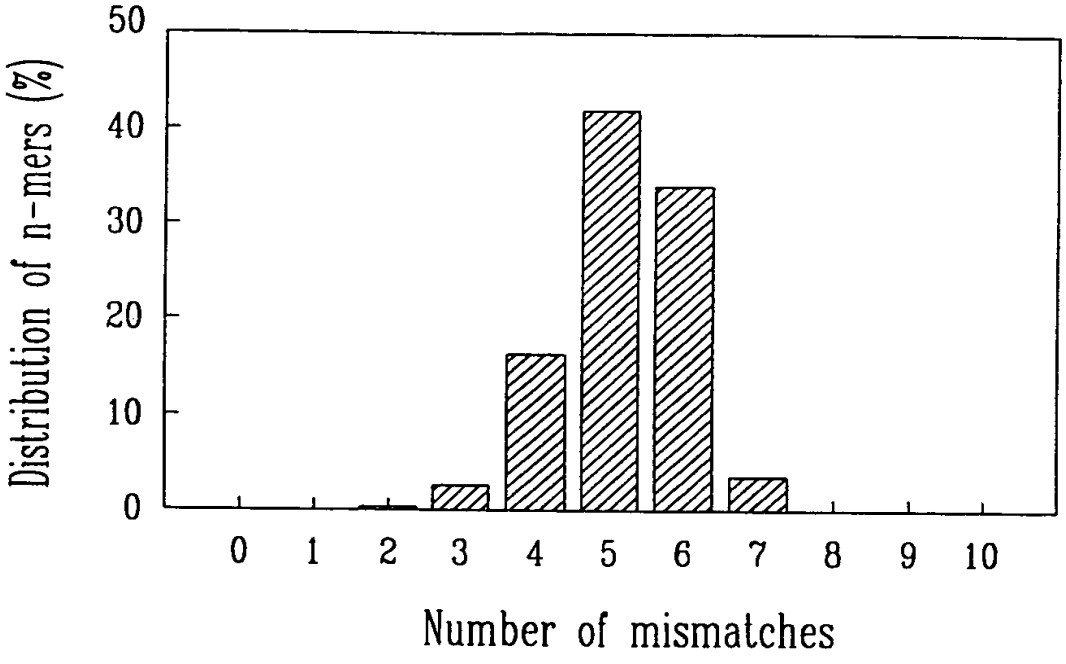
Distribution of n-mers (%)
50
40
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Number of mismatches

Fig. 6

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/CA 00/00047

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 98 15651 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUNTER (GB)) 16 April 1998 (1998-04-16) the whole document	1-15
Y	WO 98 15649 A (CREMER THOMAS ;CRAIG JEFF (DE); UNIV HEIDELBERG (DE)) 16 April 1998 (1998-04-16) the whole document	1-15
Y	SEDLACEK ET AL.: "Direct selection of DNA sequences conserved between species" NUCLEIC ACIDS RESEARCH, vol. 21, no. 15, 1993, pages 3419-3425, XP002138409 the whole document	1-15

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 May 2000	09/06/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hagenmaier, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. .ional Application No
PCT/CA 00/00047

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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